

## Effect of Bovine Somatotropin and Rumen-Undegradable Protein on Mammary Growth of Prepubertal Dairy Heifers and Subsequent Milk Production\*

A. V. Capuco,<sup>1</sup> G. E. Dahl,<sup>2,†</sup> D. L. Wood,<sup>1</sup> U. Moallem,<sup>2,‡</sup> and R. E. Erdman<sup>2</sup>

<sup>1</sup>Bovine Functional Genomics Laboratory,  
Animal and Natural Resources Institute,  
USDA-ARS, Beltsville, MD 20705

<sup>2</sup>Department of Animal and Avian Sciences,  
University of Maryland, College Park 20742

### ABSTRACT

Rapid body growth during the prepubertal period may be associated with reductions in mammary parenchymal growth and subsequent milk yield. The objective of this study was to test effects of dietary rumen-undegradable protein (RUP) and administration of recombinant bovine somatotropin (bST) during the prepubertal period on mammary growth and milk yield of dairy heifers. Seventy-two Holstein heifers were used in the experiment. At 90 d of age, 8 heifers were slaughtered before initiation of treatment. Remaining heifers were assigned randomly to 1 of 4 treatments. Treatments consisted of a control diet (5.9% RUP, 14.9% CP, DM basis) or RUP-supplemented diet (control diet plus 2% added RUP) with or without 0.1 mg of bST/kg of BW per day applied in a 2 × 2 factorial design. A total of 6 heifers per treatment (3 each at 5 and 10 mo of age) were slaughtered for mammary tissue analysis. Remaining heifers were bred to evaluate impact of treatment on subsequent milk yield and composition. Mammary parenchymal growth was not affected by RUP or bST treatment. Total parenchymal mass increased from 16 to 364 g, and parenchymal DNA from 58 to 1022 mg from 3 to 10 mo of age, respectively. Furthermore, number of mammary epithelial cells likely was not affected by diet or bST because the epithelial cell proliferation index, assessed by Ki-67 labeling, was not affected by treatment, nor was total parenchymal DNA and lipid content. Neither deleterious ef-

fects of increased rates of gain nor positive effects of bST were evident in prepubertal mammary growth. Subsequent milk production and composition was not different among treatments.

(**Key words:** heifer and mammary growth, lactation, somatotropin, protein feeding)

**Abbreviation key:** PCA = perchloric acid, RUPbST = RUP diet plus bST administration.

### INTRODUCTION

Rapid rearing of replacement dairy heifers has the potential to increase dairy profitability by bringing heifers to puberty and milk production at an early age, thus reducing the time during which the animal produces no revenue. However, rapid rearing during the prepubertal period can result in decreased milk production (Sejrsen and Purup, 1997) and more dystocia (Hoffman, 1997). Adequate skeletal size is needed to minimize dystocia during first parturition (Markusfeld and Ezra, 1993) and a positive relationship exists between BW at calving and milk production in first-lactation dairy cows (Clark and Touchberry, 1962). Maximal first-lactation milk yields occurred for Holstein replacement heifers weighing between 590 and 635 kg at calving (Keown and Everett, 1986). Others have reported that skeletal size is associated positively with first-lactation milk yield, whereas BW is associated negatively (Sieber et al., 1988; Markusfeld and Ezra, 1993).

Because the majority of skeletal growth occurs during the prepubertal period (Heinrichs and Hargrove, 1987), this period provides the greatest opportunity for enhancing skeletal growth. Although increased rates of skeletal and BW growth in prepubertal dairy heifers can be achieved by increasing the energy density of diets, increasing rates of BW gain to more than 1 kg/d reduces mammary parenchymal growth and increases mammary fat deposition (Sejrsen et al., 1982; Capuco et al., 1995), both of which may be factors associated with less milk production during the first lactation. It

Received October 27, 2003.

Accepted June 9, 2004.

Corresponding author: A. V. Capuco; e-mail: acapuco@anri.barc.usda.gov.

\*Mention of a trade name or proprietary product does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of others not mentioned.

†Present address: Department of Animal Sciences, University of Illinois, Urbana, 61801-4734.

‡Present address: Department of Dairy Cattle, Institute of Animal Science, Volcani Center, Bet-Dagan, 50250, Israel.

has been suggested that rapid rates of growth may be achieved without detrimental effects on subsequent milk production if rapid growth occurred without excessive fattening (Capuco et al., 1995; Silva et al., 2002). Additional dietary protein may prove efficacious in enabling high rates of body and skeletal gain without excessive fattening (Van Amburgh et al., 1991). Management systems that increase skeletal growth rate might be used to accelerate body growth without increasing fattening, thus preventing detrimental effects of accelerated growth on mammary development and potential effects on lactation (Kertz et al., 1987; Radcliff et al., 1997; VandeHaar, 1997; Lammers and Heinrichs, 2000).

Somatotropin, particularly when combined with increased intestinal protein (Houseknecht et al., 1992; Bruckental et al., 1997), enhanced N retention in Holstein steers, suggesting that lean tissue and skeletal growth may be improved in response to bST and additional dietary rumen-undegradable protein. Previous studies with bST showed positive effects on prepubertal growth of mammary secretory tissue (Tucker, 1987) and increased skeletal growth (Grings et al., 1990; Sejrsen, 1994; Radcliff et al., 1997, 2000). Collectively, these experiments suggest that bST in combination with added protein, provided as RUP, may be a practical means to optimize skeletal growth rates during the prepubertal period without the negative impact on mammary development.

The objectives of this study were to determine the effects of administering bST and additional dietary RUP on prepubertal growth of the mammary gland and subsequent milk production. Effects of RUP supplementation and bST administration on body composition, skeletal growth rates, and organ and tissue growth rates are the topics of companion reports (Moallem et al., 2004a; 2004b).

## MATERIALS AND METHODS

### Animal Management and Feeding

University of Maryland Institutional Animal Care and Use Committee approved the experimental protocol, and heifers were reared at the Central Maryland Research and Education Center Dairy Unit located in Clarksville, Maryland. Seventy-two Holstein heifer calves, from 2 separate groups of 36 calves (replicate blocks), were used in this experiment to evaluate the impact of prepubertal bST and RUP on body growth and composition, mammary development, and subsequent milk production. Thirty-two heifers (16 per replicate block) were slaughtered to obtain body composition data, and the remaining 40 heifers were bred and calved to provide milk production data.

Calves were raised in individual calf hutches or pens until weaning. All calves were fed colostrum for 3 d after birth and thereafter were raised on 4.5 L/d of a commercial milk replacer, ad libitum water, and starter mix until weaning at 60 d of age. After weaning, heifers were fed starter mix and water ad libitum until 90 d of age before being transitioned to a TMR fed from 3 to 10 mo. At 3 mo of age, 8 heifers were killed to determine pretreatment body composition. The remaining 64 heifers were assigned randomly to each of 4 treatments and group-fed by treatment until slaughter or onset of puberty. Twenty-four heifers, 6 per treatment (3 each at 5 and 10 mo of age), were killed to determine effects of treatment on body composition. These ages were selected to represent the midpoint of prepubertal development (5 mo) and the peripubertal period (10 mo). Treatments consisted of recombinant bST with (**RUPbST**) or without 2% added dietary RUP, applied in a  $2 \times 2$  factorial design. Sustained release recombinant bST (Posilac; Monsanto Co., St. Louis, MO), equivalent to 0.1 mg/kg of BW per d, was injected subcutaneously every 14 d in bST-treated heifers. The control diet was formulated according to 1989 NRC requirements to meet nutrient requirements including energy and protein needs for a 200-kg, large-breed heifer with a live-weight growth rate of 800 g/d (NRC, 1989).

Experimental diets were formulated to be equal in energy and RDP content, but differing in RUP content. It is important to emphasize that because the diets were not isonitrogenous, the RUP-supplemented diet contained additional crude protein supplied as RUP. The added RUP diets contained 16.9% CP, 9.0% RDP, and 7.9% RUP (DM basis), compared with 14.9% CP, 9.0% RDP, and 5.9% RUP in the control diet. Diets were fed as a TMR for ad libitum intake. Ingredient and chemical composition of diets are shown in Table 1.

Blood serum samples were collected by tail venipuncture from each heifer every 2 wk from 118 d of age until puberty. Age at puberty was assessed by serum concentrations of progesterone in blood samples (Spicer et al., 1981). After puberty, heifers from all treatments were housed and fed together according to NRC (1989) recommendations. Although the original intent was to breed heifers when they reached a BW of 385 kg, management problems resulted in delaying insemination by an average of 3 mo.

From puberty until time of breeding, heifers were fed a diet that was 23% corn silage, 75.5% alfalfa haylage, plus a 1.5% vitamin, trace-mineral supplement. The diet met or exceeded NRC (1989) nutrient requirements for 450-kg heifers with BW gain of 0.8 kg/d. From puberty until pregnancy, heifers were fed a common diet that consisted of 12.2% corn silage, 77.0% alfalfa silage, and a 1.8% vitamin, trace-mineral supplement. After

**Table 1.** Ingredient and chemical composition of control and RUP diets.

	Control	Added RUP
	% of DM	
Ingredients		
Corn grain, ground	2.3	—
Soybean meal	3.7	0.7
Excel soy <sup>1</sup>	11.3	12.8
White fish meal	—	4.7
Corn silage	64.4	64.4
Timothy hay	16.7	16.7
NaCl	0.4	0.2
Mineral and vitamin mix <sup>2</sup>	1.4	0.4
Chemical composition		
DM	46.2	46.3
CP	14.9	16.9
RUP	5.9	7.9
ADF	24.1	24.2
NDF	3.8	3.8
Ca	0.7	0.7
P	0.5	0.5

<sup>1</sup>Excel soy, Excel Soybean Technologies, Oskaloosa, KS.

<sup>2</sup>Formulated to provide (per kilogram of DM): 2551 IU of vitamin A, 426 IU of vitamin D, 8.5 IU of vitamin E, 0.06 g of P, 0.17 g of Ca, 19.2 mg of Fe, 15.4 mg of Zn, 15.4 mg of Mn, 7.7 mg of Cu, and 0.3 mg of Se.

confirmation of pregnancy, heifers were commingled with other animals in the herd and fed diets that met or exceeded NRC requirements for growth and pregnancy, but varied in ingredient content depending upon availability of forages and other feed ingredients.

Additional details regarding heifer rearing, as well as body growth and composition data, are provided in companion reports (Moallem et al., 2004a; 2004b).

### Udder Sampling and Mammary Compositional Analysis

Heifers were transported to the USDA abattoir (Beltsville Agriculture Research Center, MD), where they were slaughtered by exsanguination after stunning with a captive bolt gun. The udder was removed, trimmed of skin and teats, and separated into right and left halves. Each udder half was weighed. The right udder half was trimmed of fat based upon color of tissue and the mass of parenchyma and fat determined. Parenchyma was ground, and aliquots were frozen and stored at  $-20^{\circ}\text{C}$  until compositional analyses (DNA, RNA, protein, and lipid) were performed. In addition, samples of mammary parenchyma were obtained from the mid parenchymal region within the left rear quarter and processed for quantification of cells expressing Ki-67 nuclear proliferation antigen as subsequently described.

Nucleic acids were quantified as previously described (Capuco et al., 2001). Briefly, mammary tissue was ho-

mogenized (1:15 wt/vol) in DNA assay buffer (50 mM  $\text{Na}_2\text{PO}_4$ , 2 M NaCl, 2 mM  $\text{Na}_2\text{EDTA}$ ) using a Tekmar homogenizer (Tekmar, Cincinnati, OH). DNA was quantified using Hoechst 33258 dye binding (Labarca and Paigen, 1980) against a standard curve prepared using calf thymus DNA. Fluorescence was read using a Bio-Tek FL600 plate reader with a 360/460 nm filter set (Bio-Tek Instruments, Inc., Winooski, VT). Sample RNA was determined by ultraviolet absorbance. For this purpose, an aliquot of the above mammary homogenate was diluted with an equal volume of phosphate buffer, and perchloric acid (PCA) was added to a final concentration of 0.3 N. After incubation on ice and centrifugation, the pellet was resuspended and washed with 0.2 N PCA. The washed pellet was resuspended in 0.3 N PCA and hydrolyzed at  $37^{\circ}\text{C}$  for 60 min. Then, the concentration of PCA in the hydrolysate was increased to 0.6 N, the tube was incubated on ice, and then centrifuged. The precipitate was washed 3 times with ice-cold 0.2 N PCA. The hydrolysate and subsequent washes were combined. A portion of the collected supernatants was diluted and absorbance measured at 260 and 232 nm with a Beckman DU 650 (Beckman Instruments, Inc., Fullerton, CA).

The quantity of mammary parenchymal lipid was determined gravimetrically by chloroform-methanol extraction (Folch et al., 1957) and quantity of parenchymal protein by using the Pierce BCA protein assay (Rockford, IL) on tissue homogenates and bovine serum albumin standards.

### Immunohistochemistry

Mammary tissue samples for immunohistochemistry were fixed overnight in 10% neutral buffered formalin at  $4^{\circ}\text{C}$  and then stored in 70% ethanol until further processing. Tissues were then dehydrated through ethanol, cleared in xylene, and embedded in paraffin according to standard techniques (Luna, 1968). Tissues were sectioned at  $5\text{ }\mu\text{m}$  onto silanated slides.

The nuclear proliferation antigen, Ki-67, was detected immunohistochemically as described previously (Capuco et al., 2001). Briefly, slides were dewaxed in xylene and hydrated in a graded series of ethanol to PBS (pH 7.4). Tissue sections were quenched with 3%  $\text{H}_2\text{O}_2$  in PBS and then washed in PBS. Microwave antigen retrieval in 10 mM citrate buffer (pH 6.0) was then used. Slides were washed in PBS, blocked with 5% non-immune goat serum in PBS, and incubated overnight at  $4^{\circ}\text{C}$  with Ki-67 primary antibody (MIB-1 monoclonal antibody, Zymed Laboratories, San Francisco, CA). Cells labeled with primary antibody were stained using the Histostain SP kit (Zymed Laboratories). Slides were incubated for 30 min at room temperature with biotinyl-



ated secondary antibody, washed in PBS, and incubated with the streptavidin-peroxidase-conjugate for 10 min at room temperature. After washing in PBS, sections were incubated with diaminobenzidine, counterstained with hematoxylin or Azure II, and mounted with Permaslip (Alban Scientific Inc., St. Louis, MO).

For each tissue section, 10 randomly selected microscopic fields were photographed with a Spot digital camera (Diagnostic Instruments Inc., Sterling Heights, MI) on a Zeiss Axioskop microscope (Carl Zeiss Inc., Thornwood, NY) using a microscopic magnification of 600 $\times$ . Epithelial cells within each digital micrograph were counted and scored. At least 1000 epithelial cells were scored per heifer and the percentage of epithelial cells expressing Ki-67 nuclear proliferation antigen was determined.

### Milk Yield and Composition

Cows were milked twice daily throughout lactation and milk yield was electronically recorded at each milking for the entire lactation. Milk samples were collected monthly, alternating between a.m. and p.m. sampling milkings and analyzed for fat and protein using an infrared analyzer (Bentley Instruments, St. Paul, MN) by Lancaster DHIA (Manheim, PA).

### Statistical Analyses

Mammary growth data were analyzed by 2-way ANOVA. The Ki-67 labeling index was arcsine transformed prior to ANOVA. Bonferroni's multiple comparison test was used for post ANOVA comparisons (Prism, version 3; GraphPad Software, Inc., San Diego, CA). Milk production and component data were analyzed using the mixed models procedure in SAS (SAS Inst., Inc., Cary, NC). The statistical model included effects of bST, RUP, and RUP  $\times$  bST effects. Replicate within treatment was used as the random term to test treatment effects.

## RESULTS

Dietary RUP and bST administration successfully increased body and skeletal growth rates (see companion articles: Moallem et al., 2004a; 2004b), without producing deleterious effects on mammary growth (Table 2). Although time to puberty did not differ among treatments, RUP and bST supplementation increased BW ( $P < 0.05$ ) at puberty (Table 3), and increased or tended ( $P < 0.1$ ) to increase frame size as assessed by several measures (Table 2; Moallem et al., 2004a). From 3 until 10 mo of age, mammary parenchymal mass (right udder-half) increased from 16 to 364 g and parenchymal DNA increased from 115 to 1022 mg (data not shown).

Throughout this time, parenchymal RNA, lipid, and protein were unaffected by RUP or bST treatment, as was mammary extraparenchymal fat. The mammary epithelial cell proliferation index (Ki-67 labeling index), assessed by expression of nuclear cell proliferation antigen, did not differ among control, bST, and RUP treatments (Figure 1). Thus, prepubertal growth of mammary glands appeared to be equivalent across treatments.

At 10 mo of age, heifers in the slaughter group were killed at the predetermined time regardless of ovarian activity. Five heifers were prepubertal and 7 heifers were cycling (5 follicular phase, 2 luteal phase). Due to the limited numbers of heifers, stage of estrous cycle could not effectively be incorporated into the statistical model for mammary gland growth data. No apparent impact of stage of cycle, however, was detected on mammary epithelial growth. Across treatments, the Ki-67 labeling indices averaged  $21 \pm 2$ ,  $16 \pm 14$ , and  $21 \pm 4\%$  for follicular, luteal, and precycling heifers, respectively. Similarly, parenchymal DNA content per udder-half averaged  $1045 \pm 83$ ,  $1448 \pm 466$ , and  $826 \pm 174$  mg of DNA for follicular, luteal, and prepubertal heifers, respectively. Thus, mammary growth seemed to be similar in all treatments at 5 and 10 mo of age. Lack of effect at 10 mo did not seem to be due to an underlying influence of estrous cycle stage.

Age at first calving did not differ between treatments (Table 3) and averaged 27 mo of age. First-lactation milk yields did not differ among treatments (Table 3), averaging 9641 kg for 305-d lactation. Mean 305-d mature equivalent milk yield was 11,925 kg. Milk composition was not affected by treatment and there was no effect on 305-d fat and protein yields ( $P > 0.05$ ). Milk fat and protein averaged 3.81 and 3.01%, respectively, among all cows throughout lactation.

## DISCUSSION

This study was designed to test the hypothesis that additional dietary protein (supplied as RUP) and bST supplementation would increase prepubertal skeletal and lean body growth, and promote normal or enhanced mammary growth and subsequent milk production. Skeletal and body growth were promoted without excessive body fattening (Moallem et al., 2004a; 2004b) or deleterious effects on milk production (Table 3). Body weight and skeletal growth rates were increased at an early age by RUP addition to the diet, with smaller responses at later stages of the prepubertal period (Moallem et al., 2004a). Conversely, effects of bST on rates of BW and skeletal growth were small early, but increased as heifers matured. Effects were additive and only the RUPbST group maintained increased growth

**Table 2.** Mammary growth in heifers fed a control diet or RUP-supplemented diet, with (bST and RUPbST) or without (control and RUP) biweekly injections of bST from 90 d until puberty.

Item per right udder half	Treatment				SE <sup>1</sup>	P-value		
	Control	bST	RUP	RUPbST		bST	RUP	RUP × bST
5 mo of age								
No. of heifers	3	3	3	3				
Parenchyma, g	21.6	57.43	28.9	36.5	1.41	0.35	0.16	0.46
Extraparenchymal fat, g	121.7	116.7	162.1	104.8	23.5	0.53	0.77	0.60
Fat/parenchyma	6.16	2.32	9.29	2.72	0.59	0.06	0.48	0.58
mg Lipid/g parenchyma	182.6	145.0	147.2	173.0	16.8	0.91	0.86	0.37
mg RNA/g parenchyma	1.34	1.38	1.33	1.32	0.07	0.80	0.93	0.86
mg Protein/g parenchyma	13.78	14.61	14.12	13.13	0.52	0.91	0.81	0.83
Total parenchymal lipid, g	3.74	8.20	5.36	6.40	0.70	0.27	0.97	0.49
Total parenchymal DNA, g	0.066	0.227	0.118	0.105	0.07	0.32	0.63	0.24
10 mo of age								
No. of heifers	3	3	3	3				
Parenchyma, g	401	347	345	361	44	0.84	0.82	0.70
Extraparenchymal fat, g	555	539	880	499	68	0.18	0.32	0.22
Fat/parenchyma	1.42	2.22	2.53	1.39	0.25	0.74	0.79	0.08
mg Lipid/g parenchyma	333	380	336	408	37	0.44	0.83	0.87
mg RNA/g parenchyma	0.94	1.00	0.99	0.87	0.07	0.81	0.80	0.54
mg Protein/g parenchyma	16.5	13.9	18.6	14.6	1.2	0.57	0.20	0.77
Total parenchymal lipid, g	133	120	120	157	21	0.78	0.78	0.56
Total parenchymal DNA, g	1.05	0.93	1.01	1.10	0.13	0.95	0.81	0.70

<sup>1</sup>Standard error of the mean.

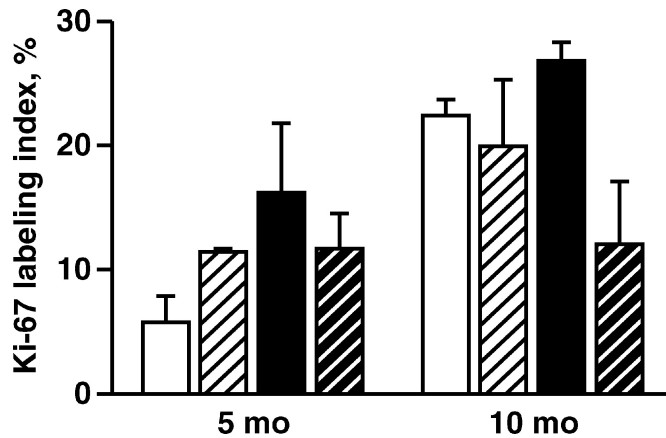
throughout the entire prepubertal period. These data suggest that the 1989 NRC recommendation for dietary protein underestimates the requirement for early postweaning heifers. Others made similar conclusions regarding the 1989 NRC recommendations because when all nutrients were increased by 15% various body growth traits were enhanced (Bortone et al., 1994). In

the current study, protein was limiting during the early postweaning period of development, whereas endogenous somatotropin may have been limiting at 250 to 300 d of age, typically the time of or just before puberty. The combination of RUP and bST increased rates of BW gain and wither height growth by 0.17 kg and 0.024 cm per d, respectively, during the treatment period (90

**Table 3.** Least square means for age and body measures at puberty, and for subsequent first-lactation performance of heifers fed a control diet or RUP-supplemented diet, with (bST and RUPbST) or without (control and RUP) biweekly injections of bST from 90 d until puberty.

Item	Treatment				SED <sup>1</sup>	P-value		
	Control	bST	RUP	RUPbST		bST	RUP	RUP × bST
Puberty								
No. of heifers	10	10	10	10				
Age, d	318	311	316	313	5.6	0.75	0.37	0.43
BW, kg	289.3	300.4	305.4	326.7	4.31	0.02	0.01	0.30
Wither height, cm	114.1	116.3	117.2	118.6	0.73	0.07	0.02	0.62
Calving age, mo	27.4	28.0	27.3	25.2	1.28	0.61	0.21	0.32
305-d yield, kg								
No. of heifers	10	10	10	10				
Milk	8931	10,355	9589	9690	512	0.12	0.99	0.18
Fat	353	372	363	356	23.9	0.27	0.92	0.84
Protein	268	310	294	387	15.6	0.23	0.89	0.10
305-d ME <sup>2</sup> yield, kg								
Milk	10,931	12,573	11,994	12,201	656	0.14	0.58	0.25
Fat	423	459	441	466	28.4	0.26	0.63	0.84
Protein	323	372	363	356	18.2	0.23	0.50	0.11
Milk composition								
Fat, %	3.94	3.71	3.72	3.88	0.17	0.83	0.88	0.23
Protein, %	3.01	3.00	3.08	2.96	0.05	0.21	0.81	0.23

<sup>1</sup>Standard error of the difference.<sup>2</sup>ME = Mature equivalent.



**Figure 1.** Effects of RUP and bST on mammary growth assessed by expression of the nuclear proliferation antigen Ki-67. The Ki-67 labeling index is expressed as a percentage of labeled mammary epithelial cells. Tissue from 3 heifers was evaluated per treatment at each slaughter age (5 and 10 mo). Treatments were control (open bars), control + biweekly bST injections (light cross-hatched bars), rumen-undegradable protein (RUP; solid bars), and RUP + bST (heavy cross-hatched bars).

d until puberty). This growth represented a 19% increase in BW gain and a 17% increase in wither height gain compared with controls (Moallem et al., 2004a).

Enhanced body and skeletal growth by RUP and bST administration before puberty did not impair mammary growth. Mammary gland mass, composition, cell numbers, and epithelial proliferation index were assessed during the mid prepubertal (5 mo) and late or peripubertal (10 mo) periods. Mammary gland mass, DNA, RNA, and protein content were unaffected by treatment, indicating equivalent mammary growth in all treatments. Extraparenchymal fat and parenchymal lipid content did not differ among treatments, indicating that mammary fat deposition, including parenchymal adipocyte content, was not influenced significantly by increased growth rate. Lack of effect on mammary tissue accretion is further supported by Ki-67 immunohistochemistry. Treatment did not affect the Ki-67 labeling index of mammary epithelial cells, indicating that the epithelial growth fraction was not altered and that rates of epithelial cell proliferation during the prepubertal period were similar across treatments. These mammary findings are in contrast to reduced mammary growth in heifers reared to achieve high rates of gain by utilizing high-energy diets (Little and Kay, 1977; Sejrsen, 1978; Sejrsen et al., 1982). The nature of hormonal mediation of decreased mammary growth is unclear (Capuco et al., 2003), but has been hypothesized to be a consequence of reduced activity of somatotropin or its mediators on the mammary gland (Sejrsen et al., 1983). Consistent with that hypothesis, IGF-I concen-

trations were elevated by dietary RUP and by bST administration (Moallem et al., 2004a).

First-lactation milk yields did not differ among treatments. Due to delays in breeding, mean age at first calving was 27 mo. Because postpubertal growth rates were similar among treatments, however, differences in body size tended to persist after puberty (Moallem et al., 2004a). Although treatment means for growth traits were not statistically different at 644 d of age, the magnitude of differences among treatment means for BW and skeletal growth parameters were analogous to those observed at 341 d of age. Thus, although not directly assessed, both RUP and bST-treated heifers should have been of larger body frame size and weight at calving. Increased prepubertal growth rates did not decrease subsequent milk yields. Milk yields for RUP or bST-treated heifers were numerically 7 to 16% greater than controls.

It has been suggested that CP is a limiting factor for developing accelerated heifer growth (Van Amburgh et al., 1991; VandeHaar, 1997). Results from the current study indicate that limiting protein is particularly problematic during the early postweaning period. By supplying a diet of high protein and energy from 4 mo of age until the luteal phase of the fifth estrous cycle, Radcliff et al. (1997) increased growth rate to 1200 g/d (controls at 800 g/d) without negatively impacting mammary development, and reduced age at puberty without hindering BW or skeletal size at puberty. Administration of bST to heifers on high-gain or control diets increased BW, skeletal size, and mammary growth (47%). In a subsequent experiment (Radcliff et al., 2000), heifers were reared on analogous diets for BW gains of 800 vs. 1200 g/d. A third group of heifers reared on the high-gain diet was injected daily with bST (25 µg/kg of BW). Heifers were bred after BW exceeded 363 kg and treatments (diet and bST) were continued until pregnancy was confirmed. Heifers in both high-gain groups were 90 d younger than control heifers at first breeding and parturition. Postpartum BW, BCS, and skeletal size did not differ among treatments. Milk production of heifers reared for high rate of gain was 14% less milk than for heifers reared at the standard rate of gain, even though the diet was formulated for high protein content. In contrast, prepubertal bST treatment prevented the decline in milk production observed in the high-gain group. In light of results from their first experiment (Radcliff et al., 1997), it was hypothesized that the high-gain group would not produce less milk than heifers in the low-gain group and that bST injection would increase milk production beyond that of heifers on the standard diet. The decline in milk production may have resulted from early breeding in the high-gain groups, but was prevented by bST admin-



istration. In the current study, RUP and bST increased skeletal and BW gains without significantly decreasing age at puberty or calving.

Lammers and Heinrichs (2000) evaluated the impact of increasing dietary protein from 11.8 to 15.6% in the diet of heifers from 7 to 12 mo of age. As in the present experiment, they observed increased skeletal growth rates with small increases in average daily gain. Effect on mammary growth was not directly evaluated and subsequent lactational performance has not been reported. However, increased teat length was interpreted as indicative of increased mammary growth with added dietary protein. In the present experiment, mammary growth was not significantly increased by RUP or bST treatments.

The relationship between heifer rearing and subsequent lactational performance is complex and the number of mammary epithelial cells at puberty seemingly does not always equate to milk production effects (Capuco et al., 2003). The current study clearly demonstrated that supplemental dietary RUP and bST administration increased heifer growth (Moallem et al., 2004a; 2004b). Such a management approach permits earlier breeding, or breeding at a constant age, but at greater BW and frame size. Mammary growth and lactational performance was similar in all treatments, suggesting that implementation of this rearing scheme might produce heifers with mammary development and milk production similar to those reared with lower rates of gain.

## CONCLUSIONS

Increased rates of body growth can be achieved in the absence of excessive fattening by appropriate nutritional management that provides sufficient protein and energy to ensure balanced growth. This may be facilitated by bST supplementation. Incorporation of additional dietary protein, supplied as RUP, and bST administration into a heifer-rearing program provided for rapid body growth and larger framed heifers, without associated decreases in prepubertal mammary development or milk production. We speculate that such regimens could be used to achieve early calving with maximal or near maximal milk production, and sufficient body size to limit dystocia. Whether RUP supplementation in heifer-rearing programs is more effective than supplementation with rumen-degradable protein remains to be rigorously demonstrated.

## REFERENCES

- Bortone, E. J., J. L. Morrill, J. S. Stevenson, and A. M. Feyerherm. 1994. Growth of heifers fed 100 or 115% of National Research Council requirements to 1 year of age and then changed to another treatment. *J. Dairy Sci.* 77:270–277.
- Bruckental, I., G. B. Huntington, C. K. Baer, and R. A. Erdman. 1997. The effect of abomasal infusion of casein and recombinant somatotropin hormone injection on nitrogen balance and amino acid fluxes in portal-drained viscera and net hepatic and total splanchnic blood in Holstein steers. *J. Anim. Sci.* 75:1119–1129.
- Capuco, A. V., R. Erdman, G. Dahl, M. Meyer, and M. Van Amburgh. 2003. Heifer Nutrition Prepubertal Growth and Development. Pages 102–115 in *Proc. 50th Maryland Nutr. Conf. Feed Manuf. and 1st Mid-Atlantic Nutr. Conf.*, Timonium, Maryland, Univ. Maryland, College Park.
- Capuco, A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad, Jr. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. *J. Dairy Sci.* 78:2709–2725.
- Capuco, A. V., D. L. Wood, R. Baldwin, K. McLeod, and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: Relation to milk production and effect of bST. *J. Dairy Sci.* 84:2177–2187.
- Clark, R. D., and R. W. Touchberry. 1962. Effect of body weight and age at calving on milk production in Holstein cattle. *J. Dairy Sci.* 45:1500–1510.
- Folch, J. M., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497–509.
- Grings, E. E., D. M. deAvila, R. G. Eggert, and J. J. Reeves. 1990. Conception rate, growth, and lactation of dairy heifers treated with recombinant somatotropin. *J. Dairy Sci.* 73:73–77.
- Heinrichs, A. J., and G. L. Hargrove. 1987. Standards of weight and height for Holstein heifers. *J. Dairy Sci.* 70:653–660.
- Hoffman, P. C. 1997. Optimum body size of Holstein replacement heifers. *J. Anim. Sci.* 75:836–845.
- Houseknecht, K. L., D. E. Bauman, D. G. Fox, and D. F. Smith. 1992. Abomasal infusion of casein enhances nitrogen retention in somatotropin-treated steers. *J. Nutr.* 122:1717–1725.
- Keown, J. F., and R. W. Everett. 1986. Effect of days carried calf, days dry, and weight of first calf heifers on yield. *J. Dairy Sci.* 69:1891–1896.
- Kertz, A. F., L. R. Prewitt, and J. M. Ballam. 1987. Increased weight gain and effects on growth parameters of Holstein heifer calves from 3 to 12 months of age. *J. Dairy Sci.* 70:1612–1622.
- Labarca, C., and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* 102:344–352.
- Lammers, B. P., and A. J. Heinrichs. 2000. The response of altering the ratio of dietary protein to energy on growth, feed efficiency, and mammary development in rapidly growing prepubertal heifers. *J. Dairy Sci.* 83:977–983.
- Little, W., and R. M. Kay. 1977. The effects of rapid rearing and early calving on the subsequent performance of dairy heifers. *Anim. Prod.* 29:131–142.
- Luna, L. G. 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. L. G. Luna, ed. McGraw-Hill Book Company, New York, NY.
- Markusfeld, O., and E. Ezra. 1993. Body measurements, metritis, and postpartum performance of first lactation cows. *J. Dairy Sci.* 76:3771–3777.
- Moallem, U., G. E. Dahl, E. K. Duffey, A. V. Capuco, and R. A. Erdman. 2004a. Bovine somatotropin and rumen-undegradable protein effects on skeletal growth in prepubertal dairy heifers. *J. Dairy Sci.* 87:3881–3888.
- Moallem, U., G. E. Dahl, E. K. Duffey, A. V. Capuco, D. L. Wood, K. R. McLeod, R. L. V. Baldwin, and R. A. Erdman. 2004b. Bovine somatotropin and rumen-undegradable protein effects in prepubertal dairy heifers: Effects on body composition and organ and tissue weights. *J. Dairy Sci.* 87:3869–3880.
- National Research Council. 1989. Page 81 in *Nutrient Requirements of Dairy Cattle*. 6th rev. ed. Natl. Acad. Sci., Washington, DC.
- Radcliff, R. P., M. J. VandeHaar, L. T. Chapin, R. E. Pilbeam, D. K. Beede, E. P. Stanisiewski, and H. A. Tucker. 2000. Effects of diet and injection of bovine somatotropin on prepubertal growth and first-lactation milk yields of Holstein cows. *J. Dairy Sci.* 83:23–29.

- Radcliff, R. P., M. J. VandeHaar, A. L. Skidmore, L. T. Chapin, B. R. Tadke, J. W. Lloyd, E. P. Stanisiewski, and H. A. Tucker. 1997. Effects of diet and bovine somatotropin on heifer growth and mammary development. *J. Dairy Sci.* 80:1996–2003.
- Sejrsen, K. 1978. Mammary development and milk yield in relation to growth rate in dairy and dual-purpose heifers. *Acta Agric. Scand.* 28:41–46.
- Sejrsen, K. 1994. Relationships between nutrition, puberty and mammary development in cattle. *Proc. Nutr. Soc.* 53:103–111.
- Sejrsen, K., J. T. Huber, and H. A. Tucker. 1983. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. *J. Dairy Sci.* 66:845–855.
- Sejrsen, K., J. T. Huber, H. A. Tucker, and R. M. Akers. 1982. Influence of nutrition on mammary development in pre- and postpubertal heifers. *J. Dairy Sci.* 65:793–800.
- Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: A review. *J. Anim. Sci.* 75:828–835.
- Sieber, M., A. E. Freeman, and P. N. Hinz. 1988. Comparison between factor analysis from a phenotypic and genetic correlation matrix using linear type traits of Holstein dairy cows. *J. Dairy Sci.* 71:477–484.
- Silva, L. F., M. J. VandeHaar, B. K. Whitlock, R. P. Radcliff, and H. A. Tucker. 2002. Short communication: Relationship between body growth and mammary development in dairy heifers. *J. Dairy Sci.* 85:2600–2602.
- Spicer, L. J., J. J. Ireland, and J. F. Roche. 1981. Changes in serum LH, progesterone, and specific binding of 125I-hCG to luteal cells during regression and development of bovine corpora lutea. *Biol. Reprod.* 25:832–841.
- Tucker, H. A. 1987. Quantitative estimates of mammary growth during various physiological states: A review. *J. Dairy Sci.* 70:1958–1966.
- Van Amburgh, M. E., D. M. Galton, D. G. Fox, and D. E. Bauman. 1991. Optimizing Heifer Growth. Pages 85–93 in *Proc. Cornell Nutr. Conf.*, Rochester, NY. Cornell Univ., Ithaca, NY.
- VandeHaar, M. J. 1997. Feeding Heifers for Lifelong Profit. Pages 101–109 in *Proc. Southwest Nutr. Man. Conf.*, Tucson, AZ. Univ. Arizona, Tucson.